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ORIGINAL ARTICLE

Phylogeographic structure in an Australian freshwater shrimp largely pre-dates the geological origins of its landscape

TJ Page and JM Hughes
Australian Rivers Institute, Faculty of Environmental Sciences, Griffith University, Nathan, Queensland, Australia

The phylogeographic structure of cryptic lineages within the freshwater shrimp *Caridina indistincta* Calman, 1926 (Decapoda: Atyidae) was investigated in an attempt to unravel any potential genetic influences of Quaternary sea-level oscillations. The study was based on mitochondrial DNA sequences from specimens from lakes and creeks in the sand dune areas of southeast Queensland, eastern Australia. Four divergent lineages were identified, two of which were from Moreton and North (N.) Stradbroke Islands. Lineage 'C1' has been found only on Moreton Island and the western part of N. Stradbroke Island, whereas 'C2' was found on the eastern side of N. Stradbroke Island and a few locations on the mainland. These diverged from each other during the Late Miocene/Pliocene and so are older than the current landscape in which they are found. Small-scale phylogeographic analysis of C1 identified four separate geographic areas, within the two islands, whose divergences date to the Pleistocene (~100–300 thousand years ago ('kya')). The N. Stradbroke Island population of C2 also diverged from the mainland during the Pleistocene, as did a sympatric freshwater fish *Rhadinocentrus ornatus* Regan, 1914 (Melanotaeniidae). This implies that the ice-age sea-level changes may have structured these populations, although there is little observable influence of the last glacial maximum (~18 kya). Most estimates for the age of the landscape (dunes, lakes) also fall within the Pleistocene and so the effect of sea-level change may be seen both in biology and geology.

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Introduction

'To try to understand present-day environments without a knowledge of their history since the last ice age would be like trying to reconstruct the plot of a long novel by reading only the last page.'

SA Elias (1995), The Ice-Age History of Alaskan National Parks (p 4)

The many 'ice ages' of the last million years have had an enormous effect on the physical landscapes of much of the world, evident in terminal moraines, glacial erratics and deep valleys. Even the sunny climes of New Guinea and Australia have hosted glaciers (Frakes *et al.*, 1987). One of the most influential effects of extreme Quaternary climate change was the many oscillations in sea level, with concomitant alterations in coastlines. This reordering of the landscape had significant flow-on effects for the plants and animals that lived within and shows that neither landscape nor biota is static. As the continental shelf drained, new land and dispersal routes opened up; and as sea levels rose, populations were sundered. This means that the imprint of the ice ages should be evident in genes as well as rocks (Hewitt, 2000).

Continental islands provide an excellent setting in which to study the biological effects of repeated sea-level change because of the intermittent nature of available dispersal routes (Bittkau and Comes, 2005). Southeast Queensland in eastern Australia has four such islands (North Stradbroke, Moreton, Bribie, Fraser) composed almost entirely of sand; the first two of which are in Moreton Bay to the east of Brisbane (Figure 1). The formation of these islands is fascinating in its own right, as their histories, like the rings of a tree, are written in the multiple, sequential sand deposits that formed during various sea-level changes of the Pleistocene (Laycock, 1978; Kelley and Baker, 1984). A combination of prevalent onshore winds from the southeast (Pickett *et al.*, 1985) and changing sea levels (Ward, 1978; Kelley and Baker, 1984) built up a series of sequential and partially overlapped parabolic dunes, each reflecting a specific period of sea-level change (Pickett *et al.*, 1985; Ward, 1985). There are fairly good correlations between dune units on Fraser, Moreton and North (N.) Stradbroke Islands (Ward, 1978; Stock, 1990; Grimes, 1992). The oldest dates obtained for sands at Cooloola (Tejan-Kella *et al.*, 1990) and sediments at Fraser Island (Longmore, 1997) are in the order of 600–730 thousand years ago ('kya').
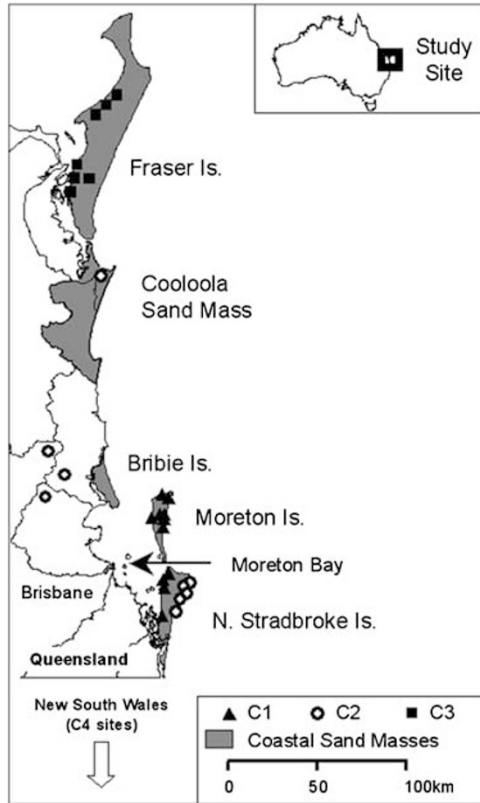
Figure 1 Sampling locations for different *C. indistincta sp.C* lineages (C1, C2, C3) in the sand dune areas of southeast Queensland, eastern Australia (see Table 1 for site details).

Moreton Bay has been a flat, broad plain during much of this time, over which many rivers flowed eastward. These rivers would have gone past large sand dune fields and continued a further 25 km to the palaeo-coastline on the continental shelf (Neal and Stock, 1986). Interglacials occurred roughly every 100 000 years (Kershaw *et al.*, 2003). When global climates warmed, the sea came flooding in and Moreton Bay became a 'drowned landscape' (Ward, 1978), isolating the composite dune fields as separate islands (as it appears today).

This area hosts the world's largest sand island (Fraser Island, 1653 km²), the highest coastal dune (Mt Tempest, Moreton Island, 280 m) and the largest concentration of perched lakes. Despite the sandy substrate, these islands are well vegetated with a mix of environments from lowland coastal heath to rainforest. Low pH freshwater lakes formed after the dunes stabilised. The water bodies are both 'water table window' lakes and swamps, which are an expression of the groundwater at the surface, and 'perched' lakes, which are set above the regional water table with their own local aquifers.

The dunes on the islands of Moreton Bay that underlie many of water bodies have been characterised as 'Awinya' (Qpa; *sensu* Ward, 1978) and 'Yankee Jack' (Qpy) (Ward, 1978; Stock, 1990). Pickett *et al.* (1985) used isotope ratios (^{230}Th/^{234}U) to date corals under Awinya sands in northwestern N. Stradbroke Island to 105 kya (revised to 119–132 kya; Tejan-Kella *et al.*, 1990). The 'Yankee Jack' dune unit overlies Awinya and so must be younger (105 kya, Kelley and Baker, 1984; ~90 kya, E Stock, personal communication).

Fully freshwater animals from insular environments are excellent model organisms to study the relationship between biota and landscape because of their dispersal limitations due to the lethal nature of intervening dry land or salt water. The freshwater shrimp *Caridina indistincta* Calman, 1926 (Decapoda: Atyidae) is widespread and common throughout the area, on all of the islands and the mainland. Chenoweth and Hughes (2003) included a few shrimp from Moreton Island in a phylogenetic study and found they represented a cryptic species (*Caridina indistincta sp.C*). In another phylogenetic study, Page *et al.* (2005) identified this species from a small number of sites in other sand areas (Cooloola Sand Mass, Fraser and N. Stradbroke Islands) and found it has large eggs, which is indicative of a fully freshwater lifecycle (Hayashi and Hamano, 1984).

A powerful method for inferring biological history, both evolutionary and biogeographic, is the phylogeographic analysis of DNA sequences, and subsequent dating of genetic divergences. This can then be related to current distributions and known geological and geomorphological history to understand their relationships. This has been done effectively with shrimp to investigate effects of Pleistocene climate change in western Queensland and Malaysia (Carini and Hughes, 2004; de Bruyn *et al.*, 2004).

The climate and sea-level changes of the last 18 000 years (last glacial maximum, 'LGM') wrought great changes on the landscapes of the world in general and Moreton Bay in particular, with sea levels 150 m below the present day level, exposing the entire continental shelf and thus providing opportunities for dispersal. This most recent glaciation and the subsequent warming and sea-level rise (17 000–6500 years ago; Neal and Stock, 1986) had significant effects on the intraspecific genetic structuring of many terrestrial and aquatic fauna throughout the world (Hewitt, 2000 and references within), including in the rainforests of Queensland (Schneider *et al.*, 1998).

Barry and Campbell (1977) considered the distribution of various terrestrial vertebrates on N. Stradbroke Island largely determined by the 'land bridge' that existed during the last glacial maximum. Given this suggestion, and the demonstrated effect of the last glacial maximum on the population structures of many taxa around the world, we hypothesise that dispersal opportunities during the LGM should have homogenised the genetic structure of freshwater taxa on these islands, especially given the islands' proximity to the mainland and the fact that they were a part of the mainland until very recently in geological and genetic terms.

A second, alternative, hypothesis is that the LGM, and the Pleistocene itself, did not have the all-encompassing influence that has sometimes been supposed. Interestingly, divergences considerably older than the last glacial maximum and the Pleistocene have also been detected in many populations whose structure had been assumed to have been determined by recent glaciation (Schneider *et al.*, 1998; Hewitt, 2000; Moritz *et al.*, 2000; Bittkau and Comes, 2005).

The two hypotheses of the overriding influence of either the LGM or the Miocene/Pliocene to explain the phylogeographic structure of *C. indistincta sp.C* on the sand islands of Moreton Bay can be tested by relating current distributions, genetic divergences, climate

history and sand dune ages. Should there be little phylogeographic structure, then the relatively recent LGM was likely most influential. Should the structuring be so deep as to predate the dune landscape, then the Miocene/Pliocene divergences seen in many east Australian freshwater taxa (see Page *et al.*, in press) may also exist within these taxa, even at such small geographic scales.

Materials and methods

Sampling
C. indistincta sensu lato were extensively sampled from every basin (135 sites in 36 basins) within an arc of 500 km emanating from Moreton and N. Stradbroke Islands, with an emphasis on these islands and adjacent mainland areas. Twenty-four sites were sampled on N. Stradbroke Island and nine on Moreton Island, and shrimp were found at nine and seven of these sites, respectively. The other sand dune areas of the region were also sampled (Fraser Island: 21 sites, Bribie Island: nine sites, Cooloola Sand Mass: four sites). Shrimp were caught with seine or dip-nets and preserved in liquid nitrogen or 95% ethanol.

DNA extraction, polymerase chain reaction and sequencing
Genomic DNA was extracted and a fragment of the mitochondrial cytochrome oxidase subunit I gene ('COI') was amplified and sequenced as per Page *et al.* (2005). All specimens were sequenced with the forward primer (CDC0.La; Page *et al.*, 2005) and a third of specimens also with the reverse primer COIa.H (Palumbi *et al.*, 1991).

Construction of data sets
A total of 426 *C. indistincta* specimens were sequenced (combined from this study, Chenoweth and Hughes, 2003; Page *et al.*, 2005 and Page and Hughes, unpublished). The only species found on Moreton Island and N. Stradbroke Island was *C. indistincta sp.C* ('Sp.C') (*sensu* Chenoweth and Hughes, 2003; Page *et al.*, 2005) and so only this taxon was used for subsequent analyses (150 Sp.C COI sequences of 450 bp). One hundred and eight of these sequences were generated for this study (new Genbank accession numbers DQ656417-DQ656436) to which were added a further eight and 34 sequences, respectively, from Chenoweth and Hughes (2003) and Page *et al.* (2005) (Table 1). All 33 unique Sp.C COI haplotypes were included in the COI phylogenetic analyses, with 10 *Caridina indistincta sp.A* ('Sp.A') haplotypes as an outgroup (some from Fraser/Bribie Islands) (Table 1). A small data set of the more conserved mitochondrial 16S ribosomal DNA ('16S') was also constructed using sequences from Page *et al.* (2005) and Page *et al.* (in press) to investigate deeper phylogenetic relationships within Sp.C. The 16S data set of 11 haplotypes of 492 bp was aligned with Sequencher 4.1.2 (Gene Codes Corporation) at default settings (Table 1).

Deep phylogenetic structure was observed within Sp.C, falling into four distinct lineages ('C1', 'C2', 'C3', 'C4'); only two of which were found on Moreton and N. Stradbroke Islands (C1, C2). For this reason, data sets for phylogeographic analyses of Moreton and N. Stradbroke Islands were split into separate sections, namely C1 (72 COI sequences) and C2 (45).

Phylogenetic analyses
The model of evolution was selected using the Akaike Information Criterion in Modeltest version 3.06 (Posada and Crandall, 1998) for both COI and 16S data sets. Three methods of phylogenetic analyses (tree building) were carried out in PAUP* version 4.0 b10 (Swofford, 2002) for each data set, namely Parsimony Analysis and Maximum Likelihood ('PA'/'ML'; both full heuristic, 100 random repetitions) and Minimum Evolution ('ME'). Each data set was bootstrapped 500 times (PA: full heuristic, 100 random repetitions; ML: 10 random repetitions). TreeRot v.2 (Sorenson, 1999) was used to calculate Bremer Support Values for both PA data sets.

Phylogeographic analyses
The relationship between local geography on Moreton and N. Stradbroke Islands and small-scale genetic structure within each lineage (C1, C2) was explored using an analysis of molecular variance (AMOVA; Excoffier *et al.*, 1992) and nested clade analysis (NCA; Templeton *et al.*, 1995). We used Arlequin version 2.0 (Schneider *et al.*, 2000) to partition genetic variation and calculate Φ-statistics given various nested geographical hierarchies in an AMOVA (islands/mainland, between islands, between areas of islands) (10 000 permutations for significance testing).

We used NCA to test whether significant associations existed between particular haplotypes and geographic locations, and further to differentiate historical and contemporary processes. A haplotype network was constructed separately for C1 and C2 using TCS version 1.21 (Clement *et al.*, 2000) and the nesting rules of Templeton *et al.* (1987) and Templeton and Sing (1993) used to assign clades. Geographical locations and haplotype nestings were entered into Geodis version 2.4 (Posada *et al.*, 2000) to test for significant associations, using the November 2005 Inference Key for NCA (available online at: Darwin.uvigo.es/download/geodisKey_11Nov05.pdf) to infer the most likely biological explanation for the significant clades.

Molecular divergence and clock calculations
COI and 16S sequence divergences between monophyletic clades were calculated using a distance matrix constructed in PAUP* using the suggested models of molecular evolution including a correction for within-clade polymorphism ($\pm$s.e.) (Avise, 1994). Divergence rates used were 1.4% per million years for COI (Knowlton and Weigt, 1998) and 0.65 and 0.9% for 16S (Sturmbauer *et al.*, 1996; Schubart *et al.*, 1998). A Likelihood Ratio Test was implemented in PAUP* to test for non-clocklike molecular evolution.

The above method is ineffective in dating divergences between areas that may share haplotypes, so we also used the coalescent method of Nielsen and Wakeley (2001) as implemented in MDIV (Nielsen and Wakeley, 2001; online at: cbsuapps.tc.cornell.edu/mdiv.aspx) to calculate the timing of population divergence (t) and expected timing of most recent common ancestor (tMRCA). We used the following MDIV parameters (model = Finite sites; [HKY], cycles = 5 000 000, burn

Table 1 Caridina sampling locations and sequences listed by lineage with Genbank accession numbers

Lineage	Island/mainland	Part of NSI/MI	Site	Site type[a]	Latitude (S)	Longitude (E)	N	COI haplotypes (N)	16S haplotypes
C. indistincta sp. C									
C1	Moreton Is.	North MI	Lake Jabiru	WTW	27° 03.16′	153° 26.14′	16	AY795016(14), DQ656423(1), DQ656426(1)	
			Spitfire Creek	CK	27° 04.29′	153° 27.04′	4	AY795016(1), AF155504(3)	AY795044
		Central MI	Blue Lagoon Campsite	CK	27° 05.41′	153° 26.70′	2	DQ656418(1), DQ656422(1)	
			Blue Lagoon	WTW	27° 05.58′	153° 26.45′	9	AY795014(6), DQ656421(1), AF155503(2)	
			Honeyeater Lake[b]	WTW	27° 05.78′	153° 26.01′	7	AY795014(7)	DQ478502
			Cravens Creek	WTW	27° 06.79′	153° 22.20′	7	AF155503(7)	
			Eagers Creek[b]	CK	27° 09.27′	153° 25.66′	4	AF155503(4)	
	N. Stradbroke Is.	Northwest NSI	Aranarawai Creek[b]	CK	27° 27.22′	153° 27.08′	5	AY795014(1), DQ656417(1), DQ656418(3)	
			Campembah Creek	CK	27° 28.11′	153° 25.50′	1	AY795015(1)	AY795045
			Brown Lake	PL	27° 29.57′	153° 25.83′	7	AY795014(7)	
		Southwest NSI	Little Canalpin Creek[b]	CK	27° 37.35′	153° 25.15′	10	DQ656419(7), DQ656420(1), DQ656427(2)	
C2	N. Stradbroke Is.	East NSI	Keyholes	WTW	27° 29.18′	153° 30.72′	5	AY795022(5)	
			Yarraman Lagoon	WTW	27° 29.22′	153° 30.62′	3	AY795022(3)	
			18-Mile Swamp	WTW	27° 31.32′	153° 29.86′	6	AY795022(6)	
			Blue Lake[b]	WTW	27° 32.06′	153° 28.86′	7	AY795022(6), AY795023(1)	AY795047
			Herring Lagoon	WTW	27° 34.55′	153° 28.16′	9	AY795022(8), DQ656425(1)	
	Mainland North (QLD)		Lake Freshwater, Cooloola	PL	25° 59.85′	153° 08.51′	11	AY795024(11)	
			Stanley River, Peachester	CK	26° 47.92′	152° 49.45′	1	AY795021(1)	
			Coochin Creek[b]	CK	26° 51′	152° 57′	1	DQ656430(1)	
			North Pine River	CK	27° 07.65′	152° 44.66′	2	AY795021(2)	AY795046
C3	Fraser Is.		Bowarrady Creek	CK	25° 07.96′	153° 09.90′	2	DQ656429(1), DQ656432(1)	
			Woralie Creek	CK	25° 11′	153° 09′	4	AY795017(1), AY795018(1), DQ656432(2)	
			Coongul Creek	CK	25° 11.83′	153° 06.64′	2	DQ656428(1), DQ656432(1)	
			Wanggoolba Creek	CK	25° 27′	152° 59′	2	AY795020(2)	
			Rocky Creek	CK	25° 28.36′	153° 00.58′	5	AY795019(2), AY795020(3)	DQ478503
			Alligator Creek	CK	25° 29.41′	152° 59.76′	2	AY795019(2)	
			Lake Birrabeen	PL	25° 30.20′	153° 02.97′	2	DQ656431(1), AY795019(1)	
C4	Mainland South (NSW)		Byron Creek at Binna Burra	CK	28° 42.55′	153° 29.81′	4	AY795027(4)	AY795049
			Nana Creek at Nana Glen	CK	30° 07′	153° 00′	4	DQ656424(3), AY795025(1)	
			Bellinger River at Bellingen	CK	30° 27.06′	152° 53.91′	4	AY795028(4)	AY795050
			Kings Creek	CK	31° 31′	152° 44′	2	AY795026(2)	AY795048
Outgroup C. indistincta sp. A									
A 'FI'a	Fraser Is.		White Lake	PL	25° 07′	153° 12	3	AY794995(3)	
			Lake Bowarrady	PL	25° 09′	153° 13′	4	AY794995(4)	
A 'FI'b	Fraser Is.		Lake Allom	PL	25° 11.91′	153° 12.63′	2	AY794993(2)	
			Ocean Lake	WTW	25° 30.20′	153° 02.97′	4	AY794993(2), AY794994(1), DQ656435(1)	DQ478499
A 'GHM'	Bribie Is.		Middle Swamp Crossing[b]	WTW	26° 57.76′	153° 07.65′	1	DQ656434(1)	
			Williams Creek, Bongaree	CK	27° 05.34′	153° 09.87′	1	DQ656433(1)	DQ478498
	Mainland North (QLD)		Coonowrin Creek[b]	CK	26° 53.92′	152° 57.64′	1	AY794997(1)	
A 'TCB'	Fraser Is.		Gerowweea Creek	CK	25° 35.94′	153° 05.07′	3	AY795000(2), DQ656436(1)	AY795038
	Fraser Is.		Govi Creek	CK	25° 35.96′	153° 05.57′	1	DQ656436(1)	
	Mainland North (QLD)		Black Swan Creek	CK	25° 47.84′	152° 52.26′	1	AF155478(1)	

Abbreviations: MI, Moreton Is.; NSI, North Stradbroke Is; QLD, Queensland; NSW, New South Wales.
[a]CK, Creek; PL, Perched Lake; WTW, Water table window lake.
[b]*R. ornatus* sequences (this study and Page *et al.*, 2004) from this site.

in $= 10\%$, M_{max} and $T_{max} =$ various) and converted the highest likelihood value for T (scaled time) and tMRCA into years using the above COI rate as described in Nielsen and Wakeley (2001).

Rhadinocentrus ornatus data set

A data set of a sympatric freshwater fish, *Rhadinocentrus ornatus* Regan, 1914 (Melanotaeniidae), was constructed to provide a comparison to the shrimp from this study. Mitochondrial ATPase sequences were obtained from a phylogenetic study (Page *et al.*, 2004). Three new fish specimens were collected and sequenced for the same gene (as per Page *et al.*, 2004) (equals accession number AY452216) from Little Canalpin Creek, N. Stradbroke Island. A haplotype network, NCA, AMOVA and MDIV analyses were carried out on the fish ATPase data set as per the shrimp COI data sets above.

Results

Phylogenetic and geographical relationships of lineages

Four distinct lineages were identified within Sp.C, namely C1, C2, C3 and C4. Both data sets and all analyses support C4 as a divergent sister group to C1/C2/C3, which form a strong clade (PA COI phylogram; Figure 2) (other bootstrap values, COI ML: 52%, COI ME: 91%, 16S PA: 90%, 16S ML: 86%, 16S ME: 82%). The relationship between C1, C2 and C3 is less clear. They form a polytomy in the COI data set, but the 16S analyses (not displayed) supports a clade of C2/C3 (bootstrap values: PA: 89%, ML: 68%, ME: 79%).

None of the lineages were found sympatrically. Lineage C4 is not only very phylogenetically divergent but is also geographically distinct, only having been located well to the south of the islands of interest, in mainland coastal basins of the state of New South Wales. Furthermore, C4 has not been found in similar sandy habitats, but largely in subtropical forest.
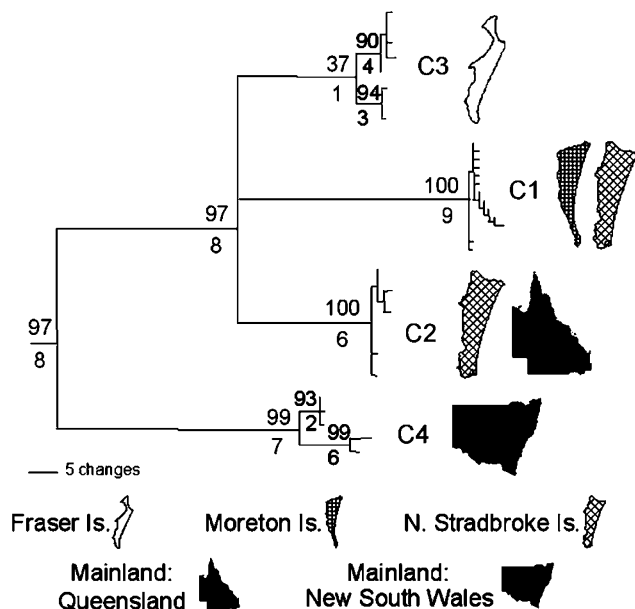

Figure 2 Parsimony phylogram of COI data set, showing specimen geographic location (Bootstrap values above node and Bremer Support values below).

The Moreton Bay islands (N. Stradbroke Island, Moreton Island) are the only known home of C1. It is the only lineage thus far found on Moreton Island, and is also on the western side only of N. Stradbroke Island (Figure 1), but not on the mainland. C2 is also found on N. Stradbroke Island, but only on the eastern side. C2 also exists in disparate populations on the mainland in the state of Queensland (Figure 1), the most significant of which is at Lake Freshwater in the Cooloola Sand Mass. C2 is also found in more rainforest-dominated environments to the south of Cooloola (Figure 1), but these appear to be small populations which are always sympatric with more numerous *C. indistincta sp.A* ('Sp.A'), which dominate this area. Very few shrimp were found on Bribie Island, and none of these were Sp.C but instead a clade of Sp.A which is common on the nearby mainland.

The final lineage, C3, has only been located on the western side of Fraser Island. There are two geographically distinct clades within C3 (Figure 1). It is unclear whether these represent separate lineages, because the area between them has not been sampled. Fraser is the only island to have both Sp.C and Sp.A (Table 1), including Sp.A 'TCB' (*sensu* Chenoweth and Hughes, 2003) which is well represented on the nearby mainland.

Divergence between lineages

All COI and 16S sequence divergences are in Table 2, with C4 notably divergent from the others. The Likelihood Ratio Test could not reject clock-like evolution in either data set (COI: $P = 0.0526$; 16S: $P = 0.2527$). Molecular clock estimates for lineage divergences (Table 2) vary considerably, but most place divergences in the late Miocene/Pliocene. These estimates should be approached with caution because of many potentially confounding factors. In particular, variation in divergence rates between lineages (Cranston and Rannala, 2005). For example, despite the COI data set passing the Likelihood Ratio Test (barely), C1 COI sequences do seem to have diverged more rapidly than the others (Figure 2). This is likely to have an effect on the deeper interlineage COI comparisons involving C1, but not the more conserved 16S comparisons, nor the shallow intralineage comparisons, where variation between individuals is much less likely. We present the various calculations in Table 2 to place divergences in a broad geological framework, rather than to date them precisely.

Population structure within C1

The network of the 14 COI haplotypes found on N. Stradbroke and Moreton Islands (Figure 3) is centred around a common haplotype, which is found on both islands. It is connected by a single mutational step to half of all the haplotypes, and is thus presumably ancestral (Templeton *et al.*, 1987). According to the NCA inference key, 'allopatric fragmentation' explains most of the significant nested clades (Clades 1–3, 1–4, 2–2, Total Cladogram; Figure 3), whereas 'restricted gene flow with isolation by distance' explains Clade 2–1, which corresponds to a linear branch of Moreton Island-only haplotypes.

When Moreton and N. Stradbroke Islands (western side) are defined as geographic groups in an AMOVA, 26.3% of the variation is partitioned between the islands,

Table 2 Genetic divergence estimates between and within lineages

Taxa	Sequence divergences		Molecular clock (mya)		MDIV (mya)		Entire range (mya)
	COI	16S	COI	16S	t	tMRCA	
C. indistincta sp. C							
C1,2,3 vs C4	18.1% (0.3)	3.0% (0.2)	12.71–13.08	3.02–4.90	7.03	8.52	3.02–13.08
C1 vs C2	12.5% (0.1)	1.9% (0.1)	8.85–8.99	2.03–3.04	4.88	5.40	2.03–8.99
C1 vs C3	9.6% (0.1)	2.6% (0.1)	6.76–6.89	2.77–4.23	3.73	4.82	2.77–6.89
C2 vs C3	5.3% (0.1)	0.9% (0.2)	3.69–3.90	0.72–1.70	2.66	3.29	0.72–3.90
C1 NMI vs CMI	0.6% (0.1)		0.34–0.48		0.30	0.57	0.30–0.57
C1 NWNSI vs SWNSI	0.2% (0.1)		0.14–0.20		0.21	0.39	0.14–0.39
C1 CMI vs NWNSI					0.11	0.33	0.11–0.33
C2 ENSI vs Main	0.3% (0.1)	0.4% (0.0)	0.13–0.25	0.47–0.65	0.23	0.42	0.13–0.65
C3a vs C3b	2.8% (0.1)		1.90–2.05		1.32	1.74	1.32–2.05
C4a vs C4b	4.1% (0.3)	1.1% (0.1)	2.77–3.15	1.02–1.83	2.16	2.56	1.02–3.15
R. ornatus							
MI/NSI vs Main					0.43	0.66	0.43–0.66
WNSI vs Main					0.35	0.66	0.35–0.66
ENSI vs MI					0.24	0.38	0.24–0.38
Within WNSI					0.07	0.10	0.07–0.10

Abbreviations: CMI: central MI; ENSI: eastern NSI; Main: mainland Queensland; MI: Moreton Is.; mya: millions of years ago; NMI: northern MI; NSI: North Stradbroke Is.; NWNSI: northwestern NSI; SWNSI: southwestern NSI; *t*: time of population divergence; tMRCA: time of most recent common ancestor; WNSI: western NSI.
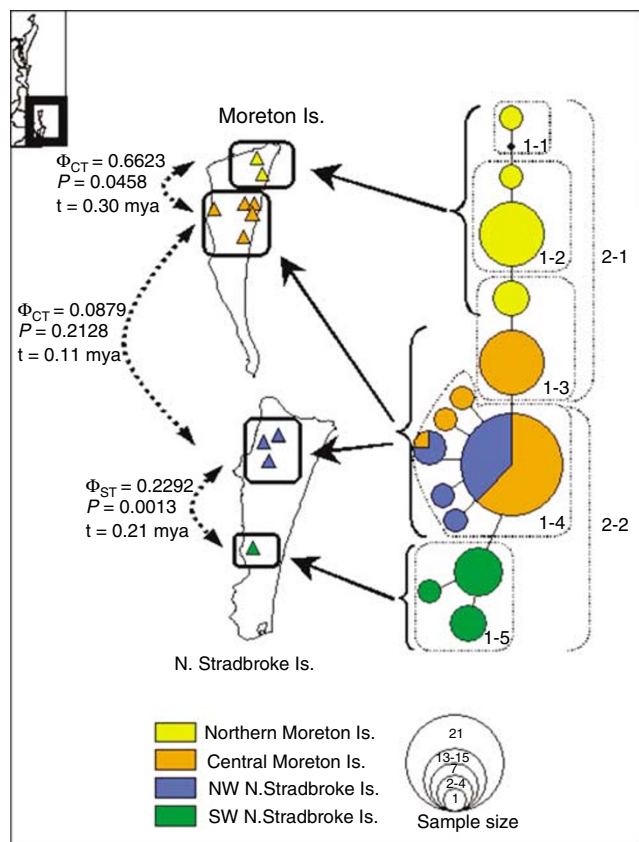

Figure 3 Phylogeography of *C. indistincta* sp.*C1* with haplotype network and NCA clades placed in local geographic context. AMOVA statistics and time since divergence (*t*) are between adjacent areas (mya = million years ago).

but with a non-significant Φ_{CT} (0.263, $P = 0.1011$). A re-examination of both the relative geographic locations (Table 1) and the haplotype network (Figure 3) makes it plain that all of the haplotypes from the northern part of

Moreton Island and the southern part of N. Stradbroke Island are unique to their respective distinct areas. In contrast, the areas encompassing the northwest of N. Stradbroke Island and the centre of Moreton Island share haplotypes (including the ancestral one) and only have haplotypes one step from the ancestral. When an AMOVA is carried out with these four areas as the groups (north Moreton Island, central Moreton Island, northwestern N. Stradbroke Island, southwestern N. Stradbroke Island), 62.58% of the variation is partitioned among them, with a high Φ_{CT} (0.6258), which is highly significant ($P = 0.0006$). Separate AMOVAs were also carried out between adjacent areas (north and central Moreton Island: 66.23%, $\Phi_{CT} = 0.6623$, $P = 0.0458$; central Moreton Island and northwest N. Stradbroke Island: 8.79%, $\Phi_{CT} = 0.0879$, $P = 0.2128$); northwest and southwest N. Stradbroke Island: $\Phi_{ST} = 0.2292$, $P = 0.0013$ (Φ_{ST} rather than Φ_{CT} because only one site in southwestern N. Stradbroke Island).

These same four groups were used to calculate time since population divergence (*t*) and time to most recent common ancestor (tMRCA) in MDIV, and all fall within the Pleistocene (Table 2). The split between north and central Moreton Island is the oldest (*t*: 0.30 mya, tMRCA: 0.57) and between central Moreton Island and north N. Stradbroke Island the most recent (*t*: 0.11 mya (millions of years ago), tMRCA: 0.33).

Population structure within C2

The mainland and N. Stradbroke Island (eastern side) share no haplotypes (Figure 4b). Clade 2–1 (mainland) is either 'allopatric fragmentation' or 'inadequate geographical sampling' depending on whether one assumes there are unsampled shrimp between the observed mainland locations (there probably are, but in low numbers). Therefore, the Total Cladogram (i.e. mainland vs N. Stradbroke Island) suffers the same fate, but 'allopatric fragmentation' is a much more likely explanation given the current presence of Moreton Bay between
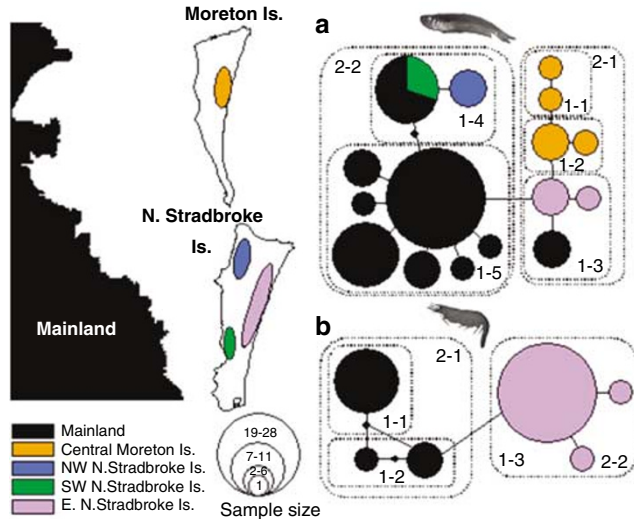
Figure 4 Nested clade analysis (NCA) of (**a**) *R. ornatus* and (**b**) *C. indistincta sp.*C2 placed in local geographic context.

them, blocking the migration of a freshwater shrimp. The Mainland/N. Stradbroke Island groups explain 80.54% of the variation in an AMOVA ($\Phi_{CT} = 0.80537$, $P = 0.0069$). MDIV dates the divergence of these groups at 0.23 mya (tMRCA: 0.42) (Table 2).

R. ornatus population structure
Moreton Island fish form a distinct branch (Clades 1–1 and 1–2 in Figure 4a), similar to the shrimp C1 (Clade 2–1 in Figure 3). In contrast, N. Stradbroke Island fish have two separate clades; one from eastern N. Stradbroke Island and related to the Moreton Island clades (1–3, and also containing a mainland haplotype), which NCA explains as 'past fragmentation and/or long distance colonization'. The other is from western N. Stradbroke Island (1–4) and is separately derived from the common mainland haplotype. Clades 1–4, 2–1 and the Total Cladogram are considered 'allopatric fragmentation' and Clade 2–2 is 'contiguous range expansion'.

An AMOVA with two groupings of Mainland and Moreton Bay Islands is not significant ($\Phi_{CT} = 0.1012$, $P = 0.1061$), but is just significant with groupings of Mainland, Moreton Island, N. Stradbroke Island ($\Phi_{CT} = 0.2457$, $P = 0.0484$), and is highly significant with Mainland, Moreton Island, eastern N. Stradbroke Island, western N. Stradbroke Island ($\Phi_{CT} = 0.4076$, $P = 0.0079$). Divergences calculated with MDIV ranged from 0.07 to 0.66 mya (Table 2).

Discussion

The LGM and the Pleistocene
If the LGM was most influential in the small-scale biogeography of these populations, there should be little genetic structure because of the short times involved (~ 18 kya). However, the LGM appears to have had little influence on the population structure of these taxa as there is very limited haplotype sharing, and a great deal of genetic structure at many levels, within and between C1 and C2.

The intralineage phylogeographic structure within both C1 (Figure 3) and C2 (Figure 4b) fall within the Pleistocene (Table 2), but well before the LGM. Comparable patterns in which there is little effect of the most recent ice-age cycle, but significant effects of earlier Pleistocene ice ages (~ 400 kya), is also visible in some European taxa (Hewitt, 2000).

Theoretically, biological histories of sympatric taxa should correlate with each other as well as with geological history given a large-scale common geological change ('comparative phylogeography'; Avise, 1994). The sympatric freshwater fish, *Rhadinocentrus ornatus*, has Pleistocene intraspecific divergences consistent with those within C1/C2 (Table 2), also considerably predating the LGM. Another sympatric fish, *Nannoperca oxleyana*, shows low levels of divergence among currently unconnected mainland creeks (Hughes et al., 1999) and thus may display the influence of palaeo-connections during the last glacial maximum. However, Moreton and Fraser Island populations of *N. oxleyana* are distinct and represent isolation from the mainland dating from earlier in the Pleistocene than the LGM.

The Miocene/Pliocene
Although Pleistocene influence, albeit not the LGM, can be observed in intralineage phylogeographic structure, it cannot account for the deeper between-lineage differences. The genetic divergences between all four *Caridina* lineages date to the Miocene/Pliocene (Table 2), when many other east Australian freshwater taxa appear to have diverged (Chenoweth and Hughes, 2003; Page et al., 2004; Ponniah and Hughes, 2006; discussed in Page et al., in press). This pattern of unexpected Miocene/Pliocene divergences has also been observed in terrestrial fauna of the wet tropics of Queensland (Schneider et al., 1998; Moritz et al., 2000), the Aegean (Bittkau and Comes, 2005) and elsewhere (Hewitt, 2000).

The C1/C2 divergence fits in well with large-scale climatic history, as Australia became colder and drier during this time (Frakes et al., 1987). However, this time-scale does not equate with the current contact zone between C1 and C2, namely the central dunes of N. Stradbroke Island that separate the east and west coasts. The genetic divergence (Table 2) is an order of magnitude older than the ages of the dunes and the water bodies (Table 3), making the shrimp far too old for their current surroundings. A similar situation has been described for stone loach in Lake Constance, as their population subdivision is much older than the lake itself (Barluenga and Meyer, 2005). Plainly these current landscapes cannot explain these levels of differentiation, and we would need to expand our scale in time and space beyond the Pleistocene and Moreton Bay to fully understand this relationship.

Colonisation history of the Moreton Bay Islands
Given that the intralineage divergences date to the pre-LGM Pleistocene, we may be able to use the Pleistocene geological history of the area to understand something of the colonisation history of its freshwater fauna. The timing of the formation of the islands, dunes and water bodies is important in unravelling the changing landscape's effect on the genetic structure of its aquatic fauna.

Table 3 Sand dune unit and approximate ages of sampling site water bodies

Part of island	Site	Caridina lineage	Site type[a]	Dune sand unit[b]	Approximate water body age[c]
North	Lake Jabiru	C1	WTW	Deflated Awinya, encroaching modern	<90±10 kya (sand), ~7.5 kya (lake)
Central	Blue Lagoon	C1	WTW	Deflated Awinya, encroaching modern	<90±10 kya (sand), ~7.5 kya (lake)
N. Stradbroke Island					
Northwest	Brown Lake	C1	PL	Reworked Awinya	<90±10 kya
Southwest	Little Canalpin Ck.	C1	CK	Cooloola dune sand	>120 kya
East	Blue Lake	C2	WTW	Eroded Awinya, encroaching Yankee	<90±10 kya

[a]CK, Creek; PL, perched lake; WTW, water table window lake.
[b]E Stock (personal communication) using Ward's (1978) units.
[c]Host sand age and lake age by encroaching sand age in kya (thousands of years).
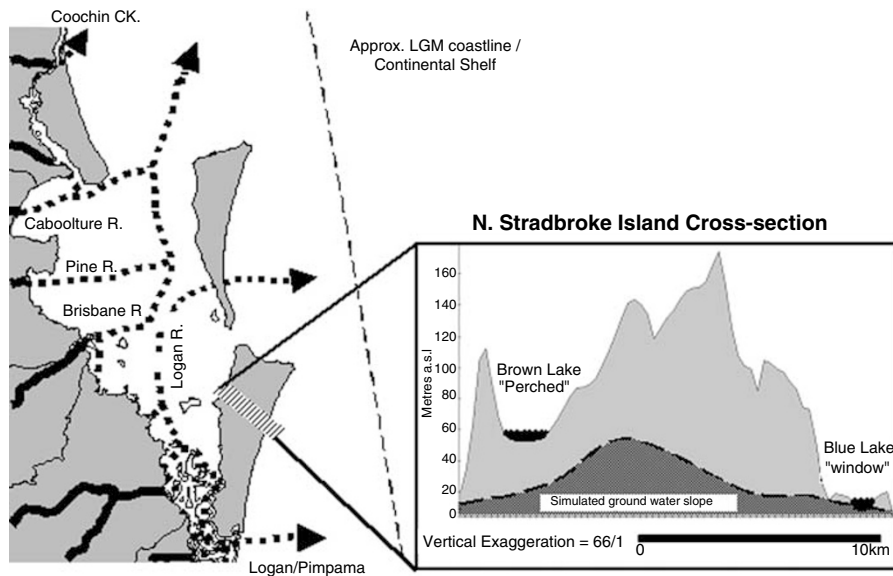
Figure 5 Palaeo-channels of southeast Queensland rivers (Jones, 1992; Lang *et al.*, 1998; Lockhart *et al.*, 1998; E Stock, personal communication), palaeo-coastline (Lang *et al.*, 1998), and cross-section of N. Stradbroke Island. Cross-section created from Dunwich Digital Topographic Data (Code: ANZQL0006000609; ID:5255; Department of Natural Resources, Mines and Water) using Profile Extractor v.6.0.

For our purpose, sand has an advantage over rock as its plasticity makes it more amenable to study at these relatively short time-scales. The precise dating of different dune unit sequences has some of the same inherent uncertainties as for molecular clock dating of DNA sequences referred to above. However, relative ages can be assigned by the stratigraphic relationships of the different dunes and the knowledge of how they formed. For example, because of the direction of prevailing winds, newer dunes tend to form on the eastern ocean side, with older dunes to the west in the centre of the islands (Laycock, 1978).

The divergences within each of the *Caridina* lineages and *Rhadinocentrus* fall within the pre-LGM Pleistocene (110–430 kya) and so are consistent with sea-level fluctuations. However, the estimates of genetic divergences (Table 2) and water body ages (Table 3) are not precise enough to correlate with specific glacial/interglacials periods. Interestingly, the population divergences within both *Caridina* and *Rhadinocentrus* tend to have a periodicity of about 100 thousand years (C1 divergences: 110, 210, 230, 300 kya; Table 2), which is also the period between major sea-level changes associated with the glacial/interglacial cycle (Kershaw *et al.*, 2003).

This implies that these divergences may have been structured by these major sea-level changes.

More precisely, how could landscape-change foster vicariance or dispersal of *Caridina* in Moreton Bay? When the plain of Moreton Bay was above sea level, the various rivers which now flow into Moreton Bay (Figure 5) would have continued past the stranded dune fields and may have provided the necessary freshwater connections between water bodies that are isolated during high sea levels (Jones, 1992; Lang *et al.*, 1998; Lockhart *et al.*, 1998). Ironically, a time of cold, arid conditions may have fostered the dispersal of a tropically derived freshwater-obligate shrimp.

This form of past connection may explain why the currently separated central Moreton and northwestern N. Stradbroke Islands are the most similar of the groups within C1, and how C2 can have colonised the eastern side of N. Stradbroke Island from the mainland without an opportunity to also colonise the west. An alternative possibility is that when C2 arrived in eastern N. Stradbroke Island, it outcompeted a previously extant population of C1. *Rhadinocentrus* also colonised each side of N. Stradbroke Island independently (Figure 4a) during the Pleistocene, emphasizing the independent colonisa-

tion history of sympatric taxa, and the independent histories of the two sides of a relatively small island. The large central dunes of N. Stradbroke Island (Figure 5) seem to have kept the different sides of the island isolated for a significant length of time.

If the rivers flowed past these dunes as recently as a few thousand years ago, why should not the *Caridina* populations be homogenised? Barry and Campbell (1977) point out that a species 'missing' from an island may be due to extinction or to a lack of opportunity to colonise. Obviously, the three dimensional structure of a landscape constrains potential paths of dispersal, especially for an obligately freshwater animal. The vagaries of chance of a river path and intervening dune may have determined whether water bodies were connected during specific glacial periods. The palaeo-channels outlined in Figure 5 may only represent the paths during the LGM and not during previous ice ages (Lang *et al.*, 1998). The aridity and low temperatures (Frakes *et al.*, 1987; Kershaw *et al.*, 2003) associated with these times likely also influenced palaeo-distributions significantly (Ponniah and Hughes, 2006).

Biological and earth histories are intimately connected and both can be reconstructed to tease out the paths they may have taken to arrive at their current positions. The 'legacy' of ice ages (Hewitt, 2000) before the most recent one is likely visible in the intraspecfic genetic structure of freshwater shrimp of Moreton Bay, whereas deep divergences dating from earlier periods of Miocene/Pliocene climate change are also present. Further interdisciplinary studies utilising both genetic and geomorphological data together should further clarify the complex relationship between landscape and biota in both time and space.

Acknowledgements

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